



# Determination of Total Phenolic Content, Analysis of Bioactive Compound Components, and Antioxidant Activity of Ethyl Acetate Seri (*Muntingia calabura* L.) Leaves from North Sumatera Province, Indonesia

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## Abstract

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**BACKGROUND:** Seri (*Muntingia calabura* L.) leaves are a plant that is often found and have not been used in various treatments even though it is reported to have various groups of bioactive compounds such as phenolic, flavonoids, tannins, saponins, steroids, and triterpenoids.

**AIM:** This study aimed to determine the total phenolic content, antioxidant activity and identify the content of potential bioactive compounds contained in the ethyl acetate fraction from *M. calabura* leaves.

**METHODS:** *M. calabura* L. leaves fraction was carried out by maceration method using ethanol followed by partition starting with *n*-hexane, chloroform, and finally ethyl acetate as solvent. The ethyl acetate fraction was continued for phytochemical screening for the content of bioactive compounds using standard reagents, determination of total phenol content by colorimetric method, determination of antioxidant activity using the DPPH method, and analysis of bioactive compounds using gas chromatography–mass spectroscopy.

**RESULTS:** The results showed that the ethyl acetate fraction of *M. calabura* leaves was positive for phenolic content which was indicated by the formation of a turquoise color after 5% FeCl<sub>3</sub> reagent was added (in ethanol), phenolic content was 0.0727 mg GAE/g dry fraction, indicating antioxidant activity (IC<sub>50</sub>) amounted to 54.437 including strong categories as antioxidants and the results of GC–MS analysis obtained various kinds of compounds and it is suspected that compounds that provide potential as antioxidants are phytol.

**CONCLUSION:** The bioactive compound of ethyl acetate fraction of seri (*M. calabura*) leaves contained phenolic components and has strong antioxidant activity.

## Introduction

Free radicals play many roles in human life. Free radicals in the body are produced from the metabolism of the ATP production process in the mitochondria. In general, the sources of free radicals are divided into reactive oxygen species free radicals and reactive nitrogen species. Low concentrations of free radicals in the body have beneficial roles, but at high concentrations, they can cause oxidative stress and damage cell walls that can trigger the emergence of various chronic and degenerative diseases such as cancer, arthritis, aging, cardiovascular, and neurodegenerative [1], [2]. Minimizing the impact and influence caused by free radicals requires an antioxidant compound. Sources of antioxidant compounds in the body are divided into endogenous antioxidants and exogenous antioxidants. Endogenous is antioxidant

compounds produced by the body while exogenous is obtained by the body through consumption of various foods such as vegetables, fruits, nuts, seeds, spices, and oils [3]. One of the potential plants that can be tested for its potency as an antioxidant is seri leaf (*Muntingia calabura* L.). The content of bioactive compounds contained in *M. calabura* leaves includes phenolics, flavonoids, tannins, triterpenes, saponins, and alkaloids [4], [5], [6]. This plant is widely found in Indonesia and grows and breeds on the side of the road and is widely used as a shade tree. Various groups of bioactive compounds contained in the plant *M. calabura* attracted the attention of researchers to determine the quantitative analysis of bioactive compounds, especially phenolics and tested their pharmacological activities as antioxidants with the 2,2-diphenyl-1-dipicrylhydrazil (DPPH) method. The previous studies have screened bioactive compounds from *M. calabura* leaves extracted with ethanol/methanol/*n*-hexane/

ethyl acetate and tested their activity. Research that examines the results of the partitioning of the ethanolic leaf extract of *M. calabura* leaves and then identified the class of compounds and the analysis, especially on the determination of bioactive compounds in a single form, is still very limited. Based on this description, the researchers were interested in determining the phenolic content of the partitioned ethanol extract of *M. calabura* leaves with ethyl acetate, which was followed by testing its antioxidant activity using the DPPH method and identifying bioactive compounds using gas chromatography–mass spectroscopy (GC–MS) as a basis which would later be used in the determination of compounds bioactive in a single form which is potential as an antioxidant.

## Materials and Methods

### Materials

The materials used include ethanol (Merck), chloroform (Merck), *n*-hexane (Merck), ethyl acetate (Merck), Folin–Ciocalteu, Whatman No 1 filter paper, Na<sub>2</sub>CO<sub>3</sub> (Merck), standard reagents for phytochemical filtration, acid gallate (Merck), FeCl<sub>3</sub> (Merck), methanol (pa), DPPH (Merck), Vitamin C (Merck), and distilled water.

### *M. calabura* sample preparation

Samples of *M. calabura* leaves were obtained from Namorambe Regency, North Sumatra Province, Indonesia, with fresh and green conditions from fruiting trees. The samples were determined and validated by a botanist with a registration number (No.5107/MEDA/2020) at the Medanense Herbarium laboratory, Universitas Sumatera Utara. The samples were cleaned with running water, drained, and then dried in a drying cabinet at a temperature of 50°C. The dry samples were ground using a kinetic blender to obtain simplicia powder. Simplicia powder is placed in a storage container and placed in a botanical laboratory before use.

### Extraction of *M. calabura* leaves bioactive compounds

*M. calabura* simplicia powder was extracted by maceration method using ethanol as a solvent at room temperature with occasional stirring with the aim of optimizing the extraction of bioactive compounds for 3 days. Once achieved, then filtered using whatman paper No. 1, so that the liquid *M. calabura* leaves ethanol extract was obtained. The residue was remacerated 2 times to optimize filtration for 2 days following the previous steps. The ethanol extract of

*M. calabura* leaves was concentrated using a vacuum rotary evaporator at 55°C and a thick extract was obtained. The viscous extract was then made graded partition based on the difference in solvent polarity, starting with separation with *n*-hexane solvent (the purpose of separating non-polar bioactive compounds), chloroform solvent (separation of semi-polar bioactive compounds), and ethyl acetate solvent (separating low polar bioactive compounds). This partition process with the liquid-liquid principle using a separating funnel then obtained the *n*-hexane extract and ethanol extract residue. Then, the ethanol extract residue was partitioned again with chloroform and then with ethyl acetate. For each treatment, the partition was repeated 3 times to maximize the partition of the bioactive compounds contained based on the polarity of the solvent. The ethyl acetate fraction obtained was concentrated using a vacuum rotary evaporator at 55°C, phytochemical screening, determination of total phenolic content, determination of activity as an antioxidant, and analysis of components of bioactive compounds using GC–MS.

### Phytochemical screening

Phytochemical screening was carried out as the first step in identifying the group of bioactive compounds contained in the ethyl acetate fraction of *M. calabura* leaves. Phytochemical screening used standard reagents against groups of compounds including flavonoids, alkaloids, saponins, phenolics, tannins, triterpenoids, and steroids [7], [8], [9], [10], [11].

### Determination of the group of total phenolic compounds

Determination of total phenolic compounds using a modified colorimetric method using Folin–Ciocalteu reagent, adding Na<sub>2</sub>CO<sub>3</sub> with a concentration of 7%, and measuring its absorbance at a wavelength of 765 nm by spectrophotometry [12], [13]. Determination of phenolic content using standard gallic acid solution with various concentrations of 50 ppm, 75 ppm, 100 ppm, 125 ppm, and 150 ppm with methanol as solvent. Each of the concentration variations was taken 200 µL, then added 1 mL of Folin–Ciocalteu and allowed to stand for 5 min followed by the addition of 4 mL of 7% Na<sub>2</sub>CO<sub>3</sub> and distilled water up to 10 mL. The solution mixture was incubated for 30 min, then the absorbance was measured at a wavelength of 765 nm. Absorbance measurements were carried out 3 times. The linear regression equation obtained is  $y = 0.0042x - 0.0468$  with  $R^2 = 0.9793$  (Figure 1). Determination of the phenolic content of the ethyl acetate fraction of *M. calabura* leaves followed the same procedure with a concentration of 1000 ppm. Phenolic content is expressed in mg by weight of gallic acid equivalent/g dry extract (mg GAE/g dry fraction).

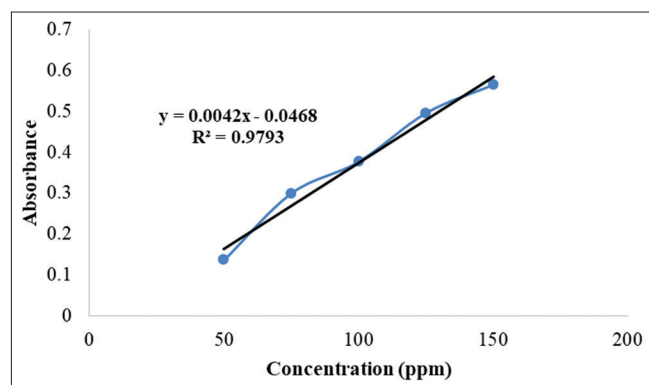


Figure 1: Gallic acid standard solution curve

### Determination of antioxidant activity

The ethyl acetate fraction of *M. calabura* leaves was carried out using the modified DPPH method. Variations in the concentration of *M. calabura* leaves ethyl acetate fraction used 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm. The volume of each concentration used was 500  $\mu$ L, then 1 mL of 0.4 mM DPPH was added and ethanol was added up to 5 mL. The mixture was incubated for 30 min. After the incubation time was reached, the absorbance was measured at 517 nm [14]. The same procedure was carried out for Vitamin C as a positive control with variations in concentrations of 6.5 ppm, 7.0 ppm, 7.5 ppm, 8.0 ppm, and 8.5 ppm. Negative control was carried out by adding 1 mL of 0.4 mM DPPH and adding ethanol to 5 mL. Absorbance measurements were carried out 3 times. Determination of free radical inhibition by the following equation:

$$\text{Inhibition (\%)} = \left[ \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}} \right] \times 100$$

Linear regression equation in determining the value of antioxidant activity conducted by plotting the percentage of inhibition curve against the concentration variations to obtain a linear regression equation. The obtained linear regression equation was used to calculate the value of antioxidant activity inhibitory concentration 50 ( $IC_{50}$ ). The  $IC_{50}$  value is expressed as the ability of the antioxidant activity of the ethyl acetate fraction leaves to reduce free radicals originating from DPPH by 50% of the initial concentration.

### Component analysis of bioactive compounds by GC-MS

The viscous fraction of *M. calabura* leaves ethyl acetate was analyzed using Thermo Scientific Trace 1310 Gas Chromatograph with column HP-5MS UI operating at an electron collision energy of 70 eV and ISQLT Quadrupole Mass Spectrometry specifications. The carrier gas used is helium with an injection volume of 0.5  $\mu$ L with a splitless injection temperature of 300°C, an ion source temperature of 280°C with a

mooring time of 5 min starting with a temperature of 100–300°C with a temperature change rate of 5°C. The identified active compounds were compared with peak retention times with the Chromeleon similarity library MS instrument.

## Results and Discussion

### Phytochemical screening

Phytochemical screening aims to obtain initial information on groups of bioactive compounds identified using standard reagents (Table 1). The results of phytochemical screening showed positive groups of flavonoid compounds, saponins, tannins, triterpenoids/steroids, and phenolics. The phenolic group was indicated by the formation of a green or blue-green color, flavonoids are indicated by the formation of a pink color, saponins are indicated by the formation of foam, tannins are indicated by the loss of bromine color, triterpenoids are indicated by the formation of a green color, and steroids are indicated by a red color. However, the ethyl acetate fraction leaves of *M. calabura* negative contained alkaloids.

Table 1: Results of phytochemical screening

| S. No. | Compound Group         | Reagents                                      | Results     |
|--------|------------------------|---|-------------|
| 1.     | Alkaloids              | Dragendorff's<br>Mayer<br>Liebermann-Bouchard | -<br>-<br>- |
| 2.     | Phenolics              | FeCl <sub>3</sub> 5% (at ethanol)             | +           |
| 3.     | Flavonoids             | Shinoda test                                  | +           |
| 4.     | Saponins               | Foaming test                                  | +           |
| 5.     | Tannins                | FeCl <sub>3</sub> 1%                          | +           |
| 6.     | Triterpenoids/steroids | Liebermann-Bouchard                           | +           |

(+) positive contains and (-) negative contains

### Determination of total phenolic content

The total phenolic content of the ethyl acetate fraction of *M. calabura* leaves from the linear regression equation was obtained  $y = 0.0042x - 0.0468$  with  $R^2 = 0.9793$  from 0.0727 mg GAE/g dry fraction. The quantitative results of this group of phenolic compounds are in line with the phytochemical screening data using FeCl<sub>3</sub>. Phenolic content is reported to show activity as an antioxidant, anticancer, anti-inflammatory, antibacterial, and xanthine oxidase inhibitor [15].

### Antioxidant activity

Antioxidant activity was determined by the DPPH method using ethanol as a solvent and measured at a maximum wavelength of 517 nm DPPH. The selection of the DPPH method in determining antioxidant activity is based on better sensitivity, relatively low cost, and simple and fast processing. The antioxidant activity value of the ethyl acetate fraction of *M. calabura* leaves expressed in  $IC_{50}$  from the linear regression equation  $y = 0.2479x + 36.505$ ;  $R^2 = 0.9859$

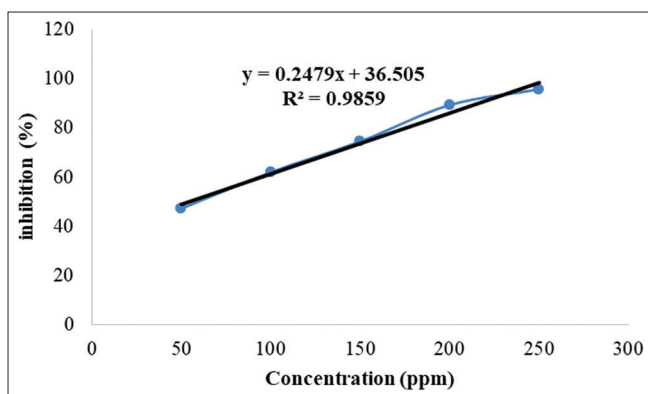


Figure 2: Test curve for the antioxidant activity

(Figure 2) of 54,437 in the strong category and  $IC_{50}$  for Vitamin C of 1.657 in the very strong category. The antioxidant activity value of the strong category of ethyl acetate fraction is supported by its ability to release protons to stabilize free radicals from DPPH to DPPH-H so as to produce neutral conditions [2]. These results are in line with the presence of phenolic content which is responsible for providing antioxidant activity.

#### GC–MS analysis activity of bioactive compounds

Analysis using GC–MS showed the content of bioactive compounds in the ethyl acetate fraction of *M. calabura* leaves. The results of the GC–MS analysis are shown in Figure 3.

The GC–MS chromatogram showed that there were 16 types of bioactive compounds with

the largest retention time span of 4.0–45.0 min. The components with the largest percentage reported that there are five bioactive compounds presented in Table 2.

The largest bioactive compound contained was phytol. Phytol is reported to have potential activity as antimicrobial, insulin enhancing, anticancer, antidiuretic [16], anti-inflammatory [17], and antioxidant [18], [19], [20]. Researchers suspect that the content of these compounds provides potential activity as antioxidants, but there are still wide opportunities for other compounds that provide synergism as antioxidants.

#### Conclusion

The ethyl acetate fraction of *M. calabura* leaves has 0.0727 mg phenolic content GAE/g dry fraction and has strong antioxidant activity. The results of the analysis by GC–MS showed the potential of phytol as an antioxidant.

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Table 2: Analysis of bioactive compounds with GC–MS

| S. No. | Compound name  | Molecular formula | $T_R$ (menit) | % area | Evidence peak |
|--------|--|-------------------|---------------|--------|---------------|
| 1.     | Phytol   | $C_{29}H_{40}O$   | 35.33         | 34.84  | 10            |
| 2.     | 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z) | $C_{19}H_{32}O_2$ | 35.11         | 3332   | 9             |
| 3.     | Hexadecanoic acid, methyl ester                        | $C_{17}H_{34}O_2$ | 31.79         | 17.77  | 7             |
| 4.     | 17-Octadecynoic acid, methyl ester                     | $C_{18}H_{34}O_2$ | 35.55         | 3.70   | 11            |
| 5.     | 7-Methyl-Z-tetradecen-1-ol acetate                     | $C_{17}H_{32}O_2$ | 23.43         | 3.39   | 8             |

GC–MS: Gas chromatography–mass spectroscopy

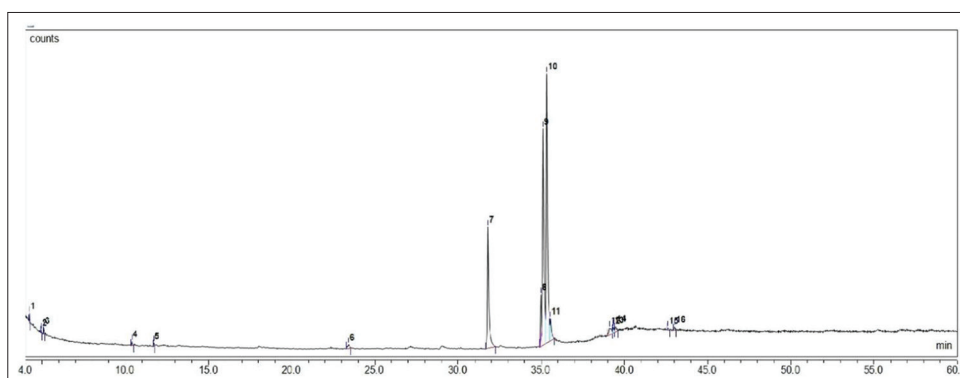


Figure 3: Gas chromatography–mass spectroscopy chromatogram *Muntingia calabura* leaves ethyl acetate fraction



and the opportunity to obtain research funds for the novice lecturer scheme to increase the ability of lecturers in research and publication.

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